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(54) Title: TREATMENT OF ACUTE MYOCARDIAL INFARCTION WITH A SUBSTANCE RELATED TO THE GROWTH HORMONE AXIS

(57) Abstract

Disclosed is the use of a substance related to the growth hormone axis, such as growth hormone (GH), growth hormone secretagogues (GHSs), e.g. growth hormone release peptide (GHRP), and insulin like growth factor I (IGF-I), or of a combination of two or more such substances, for the production of a pharmaceutical preparation for treatment of an acute ischemic event, such as an acute myocardial infarction. Also a method for treatment of an acute ischemic event, such as an acute myocardial infarction, wherein a substance related to the growth hormone axis is administered to a patient, is disclosed.

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# TREATMENT OF ACUTE MYOCARDIAL INFARCTION WITH A SUBSTANCE RELATED TO THE GROWTH HORMONE AXIS

## Background of the invention

Ischemic heart disease is the most common cause of death in both men and women in the Western world. Some of the clinical manifestations are angina pectoris, myocardial infarction, congestive heart failure (CHF) and sudden death. Despite marked improvements in the treatment including beta-blockers, trombolysis and PTCA, mortality of patients with acute myocardial infarction (MI) is still very high and almost 50% of patients surviving MI eventually develop CHF. Cardiac changes developing after MI include left ventricular dilatation and hypertrophy of the residual non-infarcted myocardium also known as remodeling. Moreover, contractile impairment often occurs after MI, clinically manifested as CHF. An alteration in myocardial energy metabolism causing energy starvation has been proposed to be an important factor for progression of CHF (see Ingwall J. S., Is cardiac failure a consequence of decreased energy reserve? Circulation 1993; 87:VII58-VII62). Using nuclear magnetic resonance (NMR) spectroscopy, it has been demonstrated that myocardial phosphor-creatinine/adenosine-tri-phosphate (PCr/ATP) ratio as an indicator of intrinsic myocardial biochemistry is a predictor of mortality in patients with CHF in late stage disease (see Neubauer S., et al., Horn M, Hahn D, Kochsiek K. Clinical cardiac magnetic resonance spectroscopy - present state and future directions. Mol. Cell. Biochem. 1998; 184:439-443).

Recent experimental and clinical studies have suggested an important role for the GH/IGF-I axis in regulation of cardiac growth and function. Cardiac function in adult patients with GH deficiency has been shown to be compromised (see Merola B., et al., Cardiac Structure and functional abnormalities in adult patients with growth hormone deficiency, J. Clin. Endocrinol. Metab. 1993;

77:1658-1661; Amato G., et al., Body composition, bone metabolism and heart structure and function in growth hormone (GH) deficient adults before and after GH replacement therapy at low doses, J. Clin. Endocrinol. Metab. 1993; 77:1671-1676; Caidahl K. et al., Cardiovascular and renal effects of growth hormone, Clin. Endocrinol. (Oxf) 1994; 40:393-400) and a recent study showing beneficial effects of GH treatment of patients with dilated cardiomyopathy has also led to the suggestion of GH as a possible future agent in the treatment of congestive heart failure (CHF)(see Fazio S., et al., A preliminary study of growth hormone in the treatment of dilated cardiomyopathy, N. Engl. J. Med. 1996; 334:809-814). However, a recently published placebo-controlled study could not confirm beneficial effects of GH on hemodynamics in patients with idiopathic dilated cardiomyopathy (see Osterziel K. J., et al., Randomised, double blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy, Lancet 1998; 351:1233-1237).

A number of experimental studies both in normal rats (see Cittadini A., et al., Differential cardiac effects of growth hormone and insulin-like growth factor-I in the rat. A combined in vivo and in vitro evaluation, Circulation 1996; 93:800-809) and in rats with impaired cardiac function (see Yang R., et al., Growth hormone improves cardiac performance in experimental performance, Circulation 1995; 92:262-267; Duerr R. L., et al., Insulin-like growth factor-1 enhances ventricular hypertrophy and function during the onset of experimental cardiac failure, J. Clin. Invest. 1995; 95:619-627; Isgaard J., et al., Growth hormone improves cardiac function in rats with experimental myocardial infarction, Eur. J. Clin. Invest. 1997; 27:517-525) show that GH and IGF-I, alone or in combination, improve contractility. Moreover, GH and IGF-I were still effective in improving cardiac function when added as an adjunct treatment to rats receiving ACE

inhibition for heart failure (Jin H., et al., Beneficial effects of growth hormone and insulin-like factor-1 in experimental heart failure in rats treated with chronic ACE inhibition, J. Cardiovasc. Pharmacol. 1995; 26:420-425). However, the absence of positive effects of GH on the impaired cardiac function after ligation of the left coronary artery (see Shen Y. T., et al., GH replacement fails to improve ventricular function in hypophysectomized rats with myocardial infarction, Am. J. Physiol. 1996; 271:H1721-H1727) and ventricular pacing of dogs (see Shen Y. T., et al., Lack of beneficial effects of growth hormone treatment in conscious dogs during development of heart failure, Am. J. Physiol. 1998; 274:H456-H466) has also been reported. It is important to note that all above mentioned studies, both basic and clinical, have been performed in a chronic state of impaired cardiac function and provides no information regarding potential acute effects of GH. In the chronic state after ischemic injury, healing with scar tissue and remodeling of the heart have occurred providing a different and at least temporarily stable situation compared to the acute phase. However, one recent study addressed more acute effects of GH after ligation of the left coronary artery in rats (see Cittadini A., et al., Growth hormone attenuates early ventricular remodeling and improves cardiac function in rats with large myocardial infarction, J. Am. College Cardiol. 1997; 29:1109-1116). Apart from induction of cardiac hypertrophy, which has been demonstrated to be a risk factor for cardiac disease, no beneficial effects of GH on systolic function could be detected by *in vivo* measurements with echocardiography. Hence, in summary, no studies have so far given any evidence of favorable GH effects in the acute phase after MI.

Until recently, clinical studies regarding GH treatment in heart failure were limited to case reports (see Cuneo R. C., et al., Cardiac failure responding to growth hormone, Lancet 1989; 1:838-839; Frustaci A., et al., Re-

versible dilated cardiomyopathy due to growth hormone deficiency, Am. J. Clin. Pathol. 1992; 97:503-511; O´Driscoll J. G., et al., Treatment of end-stage cardiac failure with growth hormone, Lancet 1997; 349:1068-1068) where GH administration dramatically improved cardiac function. In a small open study of seven patients with idiopathic dilated cardiomyopathy and CHF without GH deficiency who received GH treatment for 3 months, considerable improvement of cardiac function was reported (see Fazio S., et al., A preliminary study of growth hormone in the treatment of dilated cardiomyopathy, N. Engl. J. Med. 1996; 334:809-814). More recent studies have demonstrated beneficial effects in patients with CHF due to both ischemic and idiopathic dilated cardiomyopathy with improvements in hemodynamics when GH was added both as a maintenance therapy and as short term infusion (see Volterrani M., et al. Hemodynamic effects of intravenous growth hormone in congestive heart failure, Lancet 1997; 349:1067-1068; Beer N., et al. Beneficial effects of growth hormone in patients with congestive heart failure, Circulation 1997; 96 (suppl 1):I-521, abstract 2920). However, concern has also been raised regarding increased levels of circulating IGF-I (see Turner H., et al., Growth hormone in the treatment of dilated cardiomyopathy, Letter to the Editor, N. Engl. J. Med. 1996; 335:672-672) which may contribute to a subacromegalic condition with increased risk for hypertension, hyperinsulinemia, insulin resistance and hyperlipidemia. A possible risk for arrhythmia during prolonged GH treatment has also been pointed out (see Frustaci A., et al., Growth hormone in the treatment of dilated cardiomyopathy, Letter to the editor, N. Engl. J. Med 1996; 335:672-673). Hence, a careful dosetitration of GH and safety monitoring of patients with congestive heart failure appears to be mandatory in future clinical studies. So far, two placebo-controlled studies with GH as adjunct therapy to patients with congestive heart failure have been reported, although

neither study could confirm previously reported improvement in systolic function and lowering of walls stress. In a study by Osterziel and co-workers (see Osterziel K. J., et al., Randomised, double blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy, Lancet 1998; 351:1233-1237), fifty patients with idiopathic dilated cardiomyopathy were treated with recombinant human GH (rhGH) for three months. A significant increase in left ventricular (LV) mass was reported which was correlated to changes in serum IGF-I concentrations, although systolic function and wall stress were not affected by GH treatment. In another placebo controlled, 3 months study, rhGH treatment of patients with CHF of various etiologies was reported to be safe and well tolerated without serious adverse effects, although no significant improvement in cardiac function was seen (Isgaard J., et al., A placebo controlled study of growth hormone in patients with congestive heart failure, Eur. Heart J. 1998; 19:1704-1711). Possible explanations for the absence of cardiovascular effects in these placebo-controlled trials may be that both studies were small and that the treatment time may have been to short. Moreover, the patients in these placebo controlled studies were on a more optimal conventional therapy for congestive heart failure including higher doses of ACE inhibitors than previously described by Fazio and co-workers (see Fazio S., et al., A preliminary study of growth hormone in the treatment of dilated cardiomyopathy, N. Engl. J. Med. 1996; 334:809-814). So far, no clinical studies with acute administration of GH after myocardial infarction have been reported.

The precise mechanisms of actions of GH and IGF-I on the heart are less clear and there are few *in vitro* studies concerning with GH/IGF-I effects on cardiomyocytes. In papillary muscle from rats exposed to very high GH plasma levels due to a GH secreting tumor, it was found that the maximum $Ca^{2+}$ activated force per cross-sectional area was

increased, suggesting a GH induced elevated $Ca^{2+}$ responsiveness of the myofilaments (see Mayoux E., et al., Mechanical properties of rat cardiac skinned fibers are altered by chronic growth hormone hypersecretion. Circ Res 1993; 72:57-64). In a model with isolated buffer-perfused hearts from rats treated with high doses of GH or IGF-I for four weeks, systolic function was improved and findings supported the notion of an increased maximal response to $Ca^{2+}$ (see Strömer H., et al., Exogenously administered growth hormone and insulin-like growth factor-I alter intracellular Ca2+handling and enhance cardiac performance. In vitro evaluation in the isolated isovolumic buffer-perfused rat heart, Circ. Res. 1996; 79:227-236). However, if these effects are seen due to stimulation of cardiomyocytes rather than interstitial cells, remains to be investigated. In addition to direct effects on the heart, a lowering of arterial blood pressure peripheral resistance by GH may also be beneficial for hemodynamics (see Caidahl K. et al., Cardiovascular and renal effects of growth hormone, Clin. Endocrinol. (Oxf) 1994; 40:393-400; Jin H., et al., Beneficial effects of growth hormone and insulin-like factor-1 in experimental heart failure in rats treated with chronic ACE inhibition, J. Cardiovasc. Pharmacol. 1995; 26:420-425). It may be speculated that this effect is more rapid and could be an important factor in studies demonstrating acute cardiovascular effects of GH (see Volterrani M., et al. Hemodynamic effects of intravenous growth hormone in congestive heart failure, Lancet 1997; 349:1067-1068).

*In vitro* studies, with addition of IGF-I to cultured neonatal rat cardiomyocytes, have demonstrated changes associated with hypertrophy including increments in cell size, protein synthesis and induced expression of myosin light chain-2 and troponin I (see Ito H., et al., Insulin-like growth factor-I induces hypertrophy with enhanced expression of muscle-specific genes in cultured rat cardiomyocytes, Circulation 1993; 87:1715-1721). In adult

rat cardiomyocytes, it has been reported that IGF-I stimulates myofibril development (see Donath M. Y., et al., Insulin-like growth factor I stimulates myofibril development and decreases smooth muscle I-actin of adult cardiomyocytes, Proc. Natl. Acad. Sci. USA 1994; 91:1686-1690) and increases isometric force and free cytosolic $Ca^{2+}$ (see Freestone N. S., et al., The effect of insulin-like growth factor-1 on adult rat cardiac contractility, Molecular and Cellular Biochemistry 1996; 163/164:223-229).

Apoptosis, or programmed cell death, has been reported to occur in the myocardium of patients both after ischemic injury (see Saraste A., et al., Apoptosis in human acute myocardial infarction, Circulation 1997; 95:320-323) and in conditions with dilated cardiomyopathy (see Narula J., et al., Apoptosis in myocytes in end-stage heart failure, N. Engl. J. Med. 1996; 335-1182-1189). Some of the genes involved in the apoptotic process have been identified including the Fas-receptor which has been shown to mediate signals for apoptosis (see Itoh N., et al., The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis, Cell 1991; 66:233-243; Nagata S., et al., The Fas death factor, Science 1995; 267:1449-1456). Upregulation of the Fas-receptor has been shown to be associated with apoptosis after myocardial infarction in rats (see Kajstura J., et al., Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats, Lab. Invest. 1996; 74:86-107). It has been reported that administration of IGF-I and GH prior to cardiac ischemia attenuates the increased rate of myocyte apoptosis (see Buerke M., et al., Cardioprotective effect of insulin-like growth factor-I in myocardial ischemia followed by reperfusion, Proc. Natl. Acad. Sci. (USA) 1995; 92:8031-8035; Buerke M., et al., Human growth hormone exerts cardioprotective effects in murine reperfusion injury, Abstract # 2116, 70th Scientific Session of the American Heart Association, Orlando, November 1997). A

possible mechanism contributing to the deterioration of heart function post MI may be altered energy metabolism and a reduction of energy reserve which have been observed in rats with experimental MI (see Neubauer S., et al., Impairment of energy metabolism in intact residual myocardium of rat heart with chronic myocardial infarction, J. Clinical Invest. 1995; 95:1092-1100). However, until now the potential effects of GH on myocardial energy stores have not been evaluated.

## Summary of the invention

In the research work leading to the present invention, it was found that treatment with GH shortly after MI improves myocardial energy stores and that this improvement in myocardial energy handling is linked to an improvement in systolic function. It is reasonable to assume that normalized energy stores in the myocardium are crucial for normal cardiac function and may provide protection against further injuries. This was used as a basis to provide a new and more effective way to treat acute ischemic events, such as myocardial infarcts.

The invention thus relates to the use of a substance related to the growth hormone axis, or of a combination of two or more such substances, for the production of a pharmaceutical preparation for treatment of an acute ischemic event. The pharmaceutical preparation according to the invention should thus be administered to a patient shortly after the ischemic event first has occurred, and the sooner the better.

The invention also relates to a method for treatment of an acute ischemic event, wherein a therapeutically active amount of a substance related to the growth hormone axis is administered to a patient, preferably within a short period of time from the first manifestation of the ischemic event.

Other features, as well as advantages of the present invention will be evident from the following description and the appended claims.

## Detailed description of the invention

As stated above, the invention relates to the use of substances related to the growth hormone axis. The expression "substance related to the growth hormone axis" relates to all substances related to, linked to or involved in the sequence of successive activation reactions wherein GH is involved, comprising GH it self, hormones from the hypothalamus affecting GH and hormones or growth factors affected by GH. Preferred examples of such substances are growth hormone (GH), growth hormone secretagogues (GHSs), or insulin like growth factor I (IGF-I). Especially preferred is GH or an analogue thereof.

The growth hormone (GH) used according to the invention is preferably human growth hormone. It is possible to use both naturally derived GH and synthetically produced GH.

A growth hormone secretagogue (GHS) is a substance that stimulates secretion of GH. According to the invention it is possible to use both naturally derived GHSs and synthetically produced GH. Furthermore, the GHS used may be either a peptidic or a non-peptidic substance. One example of a peptidic GHS suitable for use according to the present invention is growth hormone release peptide (GHRP), which is a substance that leads to increased secretion of GH.

The insulin like growth factor I (IGF-I) used according to the invention is preferably human IGF-I. It is possible to use both naturally derived IGF-I and synthetically produced IGF-I.

It is also possible to use functionally equivalent analogues of GH, GHSs, or IGF-I. The expression "functionally equivalent analogue" used herein relates to any substance that is structurally similar to GH, GHSs, or

IGF-I and have essentially the same pharmacological effects.

Furthermore, it is possible to use a combination of two or more of the above mentioned substances. It is, however, not necessary to use more than one of these substances. Thus, in the most preferred embodiment only GH, or an analogue thereof, is used as the pharmaceutically active substance.

The pharmaceutical preparation produced according to the invention and the method according to the invention are suitable for treatment of ischemic events. Ischemic event is a term describing a condition with a relative shortage of oxygen supply to the myocardium manifested e.g. as angina pectoris, myocardial infarction, contractile dysfunction, arrythmia, fatigue, decreased exercise performance and breathlessness. The pharmaceutical preparation and the method according to invention are intended for treatment of acute ischemic events such as acute myocardial infarctions, angina pectoris and sudden cardiac decompensation.

The term "patient", as it is used herein, relates to any human or non-human mammal in need of treatment according to the invention.

The term "treatment" used herein relates to measures taken in order to cure or alleviate a disease or a condition.

In the method according to the present invention, a "therapeutically active amount" of the substance is used. This expression relates to a dose of the substance that will lead to the desired pharmacological effect.

The desired pharmacological effect is, as stated above, to improve the systolic function by improving the myocardial energy handling.

The pharmaceutical preparation according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants,

carriers, preservatives etc., which are well known to persons skilled in the art.

Furthermore, it is possible to combine the treatment according to the invention with conventional treatments of ischemic events.

The invention will be further illustrated in the examples below, which in no way are intended to limit the scope of the invention.

## Brief description of the drawings

In the examples below reference is made to the accompanying drawings on which:

Fig. 1 is a diagram illustrating the creatine phosphate to ATP ratio measured with $^{31}$P-MRS in MI rats treated with rhGH; the values are mean ± SEM, **p < 0.01 compared to effect of saline treatment;

Fig. 2 is a diagram illustrating the fractional shortening measured with echocardiography in MI rats treated with rhGH; the values are mean ± SEM, **p < 0.01 compared to effect of saline treatment.

## Examples

### Animals and experimental MI

The study protocol used in these examples was approved by the Animal Ethics Committee of the Göteborgs University and conducted in accordance with NIH guidelines for use of experimental animals.

The induction of MI (myocardial infarction) was performed on male Sprague-Dawley rats (B & K Universal, Sollentuna, Sweden) weighing 200-250 g, fed ad libitum and kept in 12 h light-dark cycle. All animals were maintained on standard rat pellets and tap water ad libitum. The rats were anesthetized with ketamine hydrochloride 100 mg/kg (Parke-Davis, Morris Plains, NJ, USA) and xylazine hydrochloride (Bayer AG, Leverkusen, Germany) 10 mg/kg i.p., intubated and connected to a respirator for artificial ventilation with room air and oxygen using

a Carlsson ventilator (Astra-Hässle, Göteborg, Sweden). A 2.5 cm long parasternal incision was made in the left thoracic area to the skeletal muscle. The muscle layers were separated with minimal damage using blunt dissection. Left thoracotomy was than performed between the fourth and fifth ribs, exposing the left ventricular wall. By positioning a suture between the pulmonary artery outflow tract and the left atrium, the branch of the left coronary artery was occluded. The lungs were thereafter hyperinflated, positive end-expiratory pressure was applied and the thorax immediately closed. All animals received postoperative analgesia with buprenoprin 0.05 mg/kg s.c. and 0.6 mg/100 ml in the drinking water (Temgesic, Meda, 0.3 mg/ml) and were placed in cages with temperature control for spontaneous recovery.

Echocardiography

Transthoracal echocardiography (ECHO) was used to asses left ventricular (LV) function and geometry using previously validated two-dimensional (2D), M-mode and Doppler techniques (as described by Litwin S. E., et al. in Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat, Circulation 1994; 89:345-354). The ECHO investigation was performed 24 h prior to $^{31}$P-MRS experiment, as described below, i.e. 48 h post infarct and 3 weeks later. The animals were first weighted and then anesthetized with ketamine-hydrochloride 50 mg/kg and xylazine-hydrochloride 10 mg/kg i.p. Their chests were shaved and after attachment of electrocardiographic leads they were placed prone on the left side. Two-dimensional images were obtained using commercially available ultrasound system equipped with a 10 MHz linear transducer for imaging and 5 MHz for Doppler (GE Ving Med, System five, USA). Long-axis 2D views of left ventricle including simultaneously visualization of the apex, posterior papillary muscle, aortic and mitral valves were obtained for volume measurements. LV volumes and ejection fraction

(EF) were computed using the area-length formula (see Carr K. W., et al., Measurement of left ventricular ejection fraction by mechanical cross-sectional echocardiography, Circulation 1979; 59:1196-1206). Pulsed wave Doppler spectra of mitral inflow from the apical four-chamber view were used to asses LV diastolic flow characteristics. Stroke volume (SV) was calculated using Doppler recording from the pulmonary artery (as described by Baily R. G., et al. in Non-invasive assessment of ventricular damage in rats with myocardial infarction, Cardiovasc. Res. 1993; 27: 851-855). All investigations were stored as cine-loop pictures, M-mode tracings and Doppler spectra on optical disc for off-line analysis. All measurements were averaged at least on 3 consecutive cardiac cycle using EchoPac 5.4 off-line analysis system (GE Ving Med, USA). Left ventricular meridional wall-stress (WS) was calculated from 2D measurements according to the formula: $WS = 1.33 \times P \times (Ac/Am) \times 10^3$ dynes/cm$^2$ (see Douglas P. S., et al., Estimation of wall stress and left ventricular mass by noninvasive techniques and clinical implications, Cardiovasc. Clin. 1986; 17:103-128; Douglas P. S., et al., Comparison of echocardiographic methods for assessment of left ventricular shortening and wall stress, J. Am. Coll. Cardiol. 1987; 9:945-951). In the formula, P is the systolic blood pressure, Am is the myocardial area determined by subtraction of the LV cavity area (Ac) from the total LV area and 1.33 is conversion constant from millimeters of mercury to dynes/cm$^2$.

Echocardiographic estimation of infarct size

The size of MI was estimated according to the score system as described by Baily R. G. (supra). The left ventricle was arbitrary divided into 4 regions in LAX and SAX. Each of the segments in parasternal LAX and SAX views was assigned a point for the presence of akinesis and/or dyskinesis. Additional point was assigned for the presence of LV dilatation on the M-mode tracing. Dilatation was defined as 15% more than the average of the left

ventricular internal diameter (LVDd) in end diastole of the control group. A myocardial infarction was considered to be small if the total score was 1-2 points, moderate if 3 or 4 and large if ≥ 5 points. Previous study has shown that infarcts with ≥ 5 points affected at least 35% of the LV. Based on the results from this score criteria only the rats with large infarcts and EF < 45 % were selected for treatment randomization. Animals, which on ECHO did not shown signs of myocardial infarction, were defined as sham operated.

It has been shown previously that MI > 1/3 of LV is associated with progressive LV remodeling and hemodynamic abnormalities characteristic for heart failure while MI < 1/3 of LV or nontransmural infarcts do not consistently cause this changes (see Pfeffer M. A., et al., Myocardial infarct size and ventricular function in rats, Circ. Res. 1979; 44:503-512). The inclusion criteria for rats with MI were that > 1/3 of the LV circumference was showing signs of irreversible ischemia.

In vivo $^{31}$P-magnetic resonance spectroscopy (MRS) of the rat heart

MR imaging and volume-selective cardiac $^{31}$P-MRS were performed on a 2.35 Tesla (T) horizontal magnet with a 20 cm bore (Bruker Biospec 24/30) according to the method previously described by our laboratory. The magnetic field homogeneity was optimized until line width of the water $^{1}$H-signal at half height was < 0.5 ppm. A surface coil of 5 cm diameter dual tuned ($^{1}$H and $^{31}$P) was used for radio frequency transmission and reception.

Rats were given induction anesthesia with fentanyl/fluanizon (Hypnorm, Janssen) 0.5 mg/kg and diazepam (Stesolid) 2.5 mg/kg. Constant body temperature (36.5 ± 0.5C°) was maintained by specially adapted homeothermic blanket system (Harvard Apparatus). The animals were placed with heart region lying on the surface coil in prone position to minimize respiratory movements of the chest wall. The paws were wrapped in thin copper foil and

connected via carbon electrodes to the Physiogard SM 785 MR monitoring system (Bruker, Karlsruhe, Germany). Continuous ECG signal was then acquired and used for synchronization of RF pulses and monitoring of HR. The animals were maintained anaesthetized by continuous gas anesthesia with isoflurane delivered in the mixture of oxygen and nitrous oxide (1:1) in the concentration 0.4-1 % and at flow rate 0.4 l/min.

A gradient echo imaging method was used for visualization of the heart and selection of VOI for $^{31}$P-MRS. All images as well as spectroscopic data acquisition were obtained with synchronized RF pulses to the cardiac rhythm with triggering delay of 1 ms after the R wave. Five images (2.5 mm slice thickness and field of view of 8 cm) were acquired in the sagittal plane in order to visualize the thoraco-abdominal region and the heart. Starting from the scout image in the sagittal plane 5 additional (2.5 mm) images were taken perpendicular on the LV long axis. Image selected in vivo spectroscopy (ISIS) (see Ordridge R. J., et al., Image-Selected In vivo Spectroscopy (ISIS): a new technique for spatially selective NMR spectroscopy, J. Magn. Reson. 1985; 66:283-294) was employed for spatial localization. The size of the VOI was 10 x 12 x 14 $mm^3$ (1.68 $cm^3$) which included as much of the LV as possible. Selection of the VOI was made first on the images in the sagittal plane and then the position was cross-checked on the images in the transverse plane. If the VOI included parts of chest muscles, diaphragm or liver it was repositioned. Accumulation parameters were 512 scans, 4k data points and 2500 Hz sweep width with a 4.5 s repetition time giving the total scan time 38 min. Spectroscopic processing consisted of Fourier transformation followed by first order phase correction, Lorenzian curve fitting and integration of PCr, ß-ATP, 2,3-DPG + and Pi peaks. Phosphocreatine, ATP, and 2,3-DPG were calculated by computer integration of the areas under the respective peaks. Myocardial PCr/ATI ratio was corrected

for partial saturation for each animal separately by use of the correction factor calculated from PCr/ATP measured from unlocalized spectra of the chest region acquired at 4.5 s and 15 s repetition time (see Bottomley P. A., Correcting human heart 31P NMR spectra for partial saturation. Evidence that saturation factors for PCr/ATP are homogenous in normal and disease states, Journal of Magnetic Resonance 1991; 95:3). After completion of the second $^{31}$P-MRS examination, the rats were sacrificed by rapid excision of the heart. The mass of LV + RV without atria, LV + infarct and RV were measured separately.

Statistics

Computer software (Statview 4.57) was used to perform standard statistical procedure with calculation of mean value and standard deviation (SD) in the different groups. Fisher's PLSD (Protected Least Significant Difference) test preceded by one-way analysis of variance (ANOVA) was applied. Normal distribution of the data was assessed with Kolmogorov - Smirnov test. When data were not normally distributed, non-parametric test was applied (Kruskal-Wallice). Simple correlation was examined by regression analysis. The value $p < 0.05$ was considered as statistically significant. All data are presented as mean ± SEM, unless otherwise indicated.

Results

It was found that the PCr/ATP ratio measured by $^{31}$P-MRS was not significantly different between the placebo and the GH treated groups at baseline, although both groups had significantly lower PCr/ATP ratio compared to the sham group. This is shown in Figure 1. After 3 weeks of treatment, the PCr/ATP ratio was significantly increased compared to the placebo group, which illustrates that the cardiac energy status is normalized after treatment with GH.

The fractional shortening (FS) measured by echocardiography was not significantly different between placebo and the GH treated group at baseline, although both

groups had significantly lower FS compared to the sham group. This is illustrated in Figure 2. After 3 weeks of treatment, the FS was significantly increased compared to the placebo group where a further deterioration was seen.

CLAIMS

1. Use of a substance related to the growth hormone axis, or of a combination of two or more such substances, for the production of a pharmaceutical preparation for treatment of an acute ischemic event.

2. Use according to claim 1, wherein said pharmaceutical preparation improves myocardial energy stores.

3. Use according to claim 2, wherein said improvement is a normalization of the myocardial energy stores.

4. Use according to any one of the claims 1-3, wherein said substance is growth hormone (GH), or a functionally equivalent analogue thereof.

5. Use according to any one of the claims 1-3, wherein said substance is a growth hormone secretagogue (GHS), or a functionally equivalent analogue thereof.

6. Use according to claim 5, wherein said substance is growth hormone release peptide (GHRP), or a functionally equivalent analogue thereof.

7. Use according to any one of the claims 1-3, wherein said substance is insulin like growth factor I (IGF-I), or a functionally equivalent analogue thereof.

8. Use according to anyone of the claims 1-7, wherein said ischemic event is an acute myocardial infarction.

9. Use according to anyone of the claims 1-7, wherein said ischemic event is angina pectoris.

10. Use according to anyone of the claims 1-7, wherein said ischemic event is sudden cardiac decompensation.

11. A method for treatment of an acute ischemic event, wherein a therapeutically active amount of substance related to the growth hormone axis is administered to a patient.

12. A method according to claim 11, wherein said substance upon administration results in improvement of the myocardial energy stores.

13. A method according to claim 12, wherein said improvement is a normalization of the myocardial energy stores.

14. A method according to any one of the claims 11-13, wherein said substance is growth hormone (GH), or a functionally equivalent analogue thereof.

15. A method according to any one of the claims 11-13, wherein said substance is a growth hormone secretagogue (GHS), or a functionally equivalent analogue thereof.

16. A method according to claim 15, wherein said substance is growth hormone release peptide (GHRP), or a functionally equivalent analogue thereof.

17. A method according to any one of the claims 11-13, wherein said substance is insulin like growth factor I (IGF-I), or a functionally equivalent analogue thereof.

18. A method according to anyone of the claims 11-17, wherein said ischemic event is an acute myocardial infarction.

19. A method according to anyone of the claims 11-17, wherein said ischemic event is angina pectoris.

20. A method according to anyone of the claims 11-17, wherein said ischemic event is sudden cardiac decompensation.

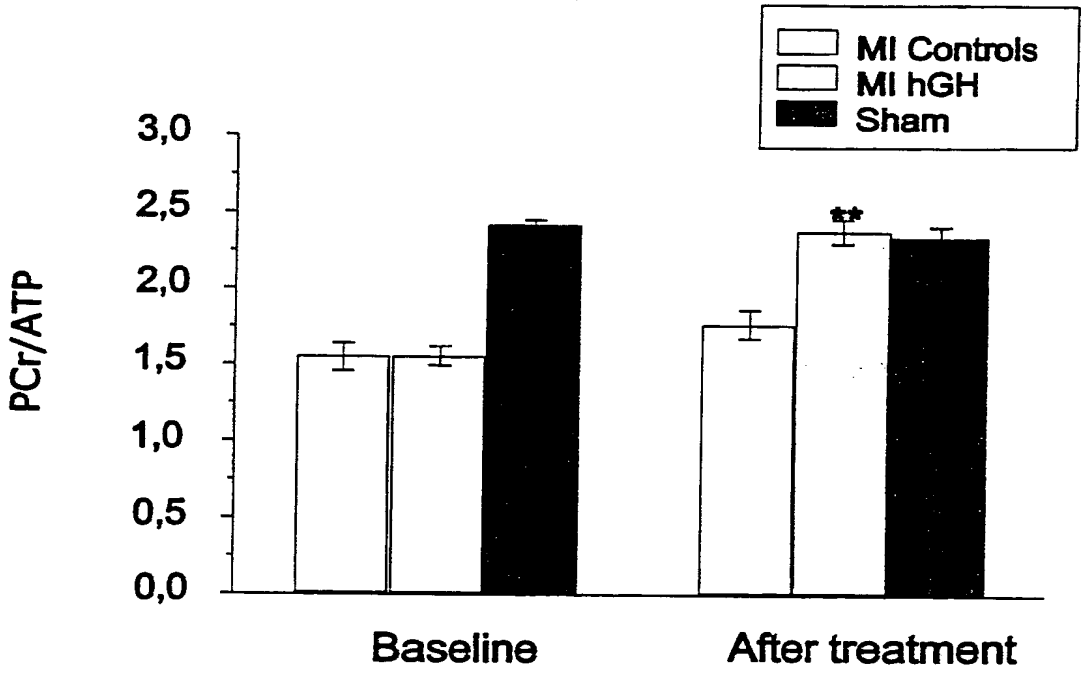
MI Controls
MI hGH
Sham
PCr/ATP
3,0
2,5
2,0
1,5
1,0
0,5
0,0
**
Baseline
After treatment

Fig. 1

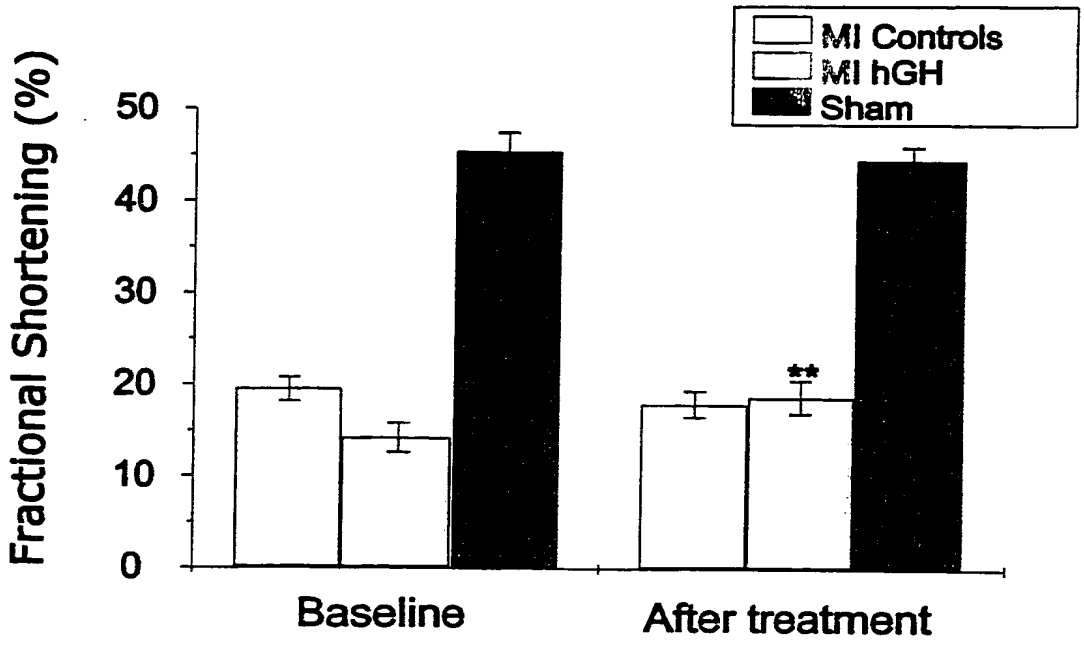
MI Controls
MI hGH
Sham
Fractional Shortening (%)
50
40
30
20
10
0
**
Baseline
After treatment

Fig. 2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00844

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/00, A61K 38/27, A61K 38/30, A61P 9/10
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | International Journal of Cardiology, Volume 69, 1999, Hugo E. Castagnino, "Great expectations from a different approach to the treatment of acute myocardial infarction: cytoprotection", page 15 - page 18, see esp. page 17, left column, lines 33-43 -- | 1-20 |
| X | Miner Electrolyt Metab, Volume 25, No 1-2, 1999, Cittadini A et al, "Growth hormone and the heart", page 51 - page 55, see esp. page 53 -- | 1-20 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 4 Sept 2000 | 08 -09- 2000 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. + 46 8 666 02 86 | Authorized officer<br>Hampus Rystedt/EÖ<br>Telephone No. + 46 8 782 25 00 |

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00844

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | International Journal of Cardiology, Volume 35, 1992, H.E. Castagnino et al, "Preservation of the myocardial collagen framework by human growth hormone in experimental infarctions and reduction in the incidence of ventricularaneurysms" page 101 - page 114 | 1-6,8-16, 18-20 |
| X | WO 9211865 A1 (KABI PHARMACIA AB), 23 July 1992 (23.07.92), see abstract, claims | 1-3,7-13, 17-20 |
| X | International Journal of Cardiology, Volume 59, 1997, M. Scheinowitz et al, "The role of insulin-like and basic fibroblast growth factors on ischemic and infarcted myocardium: a mini review", page 1 - page 5, see sections 4-8 | 1-3,7-13, 17-20 |
| A | WO 9528174 A1 (GENENTECH, INC.), 26 October 1995 (26.10.95), see abstract, claims | 1-20 |
| A | Horm Metab Res, Volume 31, No 2-3, 1999, Isgaard J et al, "The role of the GH/IGF-I axis for cardiac function and structure" page 50 - page 54 | 1-20 |
| A | WO 9901151 A1 (PHARMACIA & UPJOHN AB), 14 January 1999 (14.01.99) | 2,3,12,13 |
| A | Endocr J, Volume 43, Suppl:S. October 1996, Ho KK et al: "Metabolic actions of growth hormone in man", page 57 - page 63 | 2,3,12,13 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00844

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 11-20
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet (1)

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet (2)

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00844

1.Claims 11-20 relate to methods for therapeutic treatment of the human or animal body, see Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

2. According to Article 6 PCT, the claims should be fully supported by the description. The present claims are not considered to fulfill this requirement for the following reasons: The expression "a substance related to the growth hormone axis" as defined on page 9 is very broad. It includes a large number of both known and unknown compounds and probably also compounds which are presently not known to be related to the growth hormone axis. It is therefore not possible to perform a search over the whole scope of the claims. Furthermore, the description only provides experimental support for the use of growth hormone itself in the invention. The description does not provide any support for the assumption that normalized myocardial energy stores lead to the beneficial effects claimed, but only a statement that this seems reasonable (page 8, lines 16-19).

The search has consequently been restricted to the searchable features: The use of growth hormone or IGF-1 in the treatment of acute myocardial infarction, angina pectoris and sudden cardiac decompensation.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 00/00844

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | SE 9802023 D | 00/00/00 |
| | | WO 9964062 A | 16/12/99 |

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